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☐	L1	enzymatic$ near amplif$	3831
☐	L2	L1 same (target-probe complex or target-probe moiety or (target near probe) or locator probe)	8
☐	L3	L1 and ((probe near target complex) or (probe near target moiety))	60
☐	L4	L3 and (extension near nucleic acid)	0
☐	L5	L3 and (extension near (nucleic acid or primer or oligonucleotide or probe or polynucleotide))	43
☐	L6	L5 and (hybridiz$ near (nucleic acid or probe or primer or oligonucleotide or polynucleotide))	43
☐	L7	L6 and polymerase	42
☐	L8	L7 and exonuclease	25
☐	L9	L8 and (label$ same (biotin or FAM or carboxyfluorescein or HEX or hexachlorofluorescein or tetrachlorofluorescein or ROX or carboxy rhodamine or TAMRA or JOE))	18
☐	L10	L9 and detection probe	2

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L2 4322 ENZYMATIC? (3A) AMPLIFI?

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L3 1187 L2 AND PROB?

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L4 596 L3 AND HYBRIDIZ?

=> s l4 and extension
L5 16 L4 AND EXTENSION

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 16 DUP REM L5 (0 DUPLICATES REMOVED)

=> d ibib abs 1-16

L6 ANSWER 1 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-09694 BIOTECHDS
TITLE: Enriching low abundance polynucleotide relative to a high abundance polynucleotide in a sample, for analyzing gene expression and creating cDNA libraries, comprises blocking polymerase activity on high abundance polynucleotides; low abundance polynucleotide for use in gene expression analysis
AUTHOR: SCHROEDER B G; CHEN C; SCHROTH G P
PATENT ASSIGNEE: SCHROEDER B G; CHEN C; SCHROTH G P
PATENT INFO: US 2004014105 22 Jan 2004
APPLICATION INFO: US 2003-435489 9 May 2003
PRIORITY INFO: US 2003-435489 9 May 2003; US 2002-144179 9 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-121562 [12]
AN 2004-09694 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Enriching low abundance polynucleotide relative to a high abundance polynucleotide in a sample, where the ratio of the high abundance polynucleotide to the low abundance polynucleotide is at least 10:1, comprising blocking polymerase activity on high abundance polynucleotides by using enzymatically non-extendable nucleobase oligomers, is new.
DETAILED DESCRIPTION - Enriching low abundance polynucleotide relative to a high abundance polynucleotide in a sample, where the ratio of the high abundance polynucleotide to the low abundance polynucleotide is at least 10:1, comprising: (a) exposing the sample to at least one first enzymatically non-extendable nucleobase oligomer having a

nucleobase sequence complementary to a sequence within the high abundance polynucleotide under conditions such that base pairing occurs; (b) exposing the sample to a primer having a nucleobase sequence complementary to a sequence within the low abundance polynucleotide under conditions such that base pairing occurs; and (c) subjecting the sample to conditions for polymerase **extension**, so that the low abundance polynucleotide is amplified by **extension** of the primer and the high abundance polynucleotide is not amplified. INDEPENDENT CLAIMS are also included for the following: (1) polynucleotides, where the relative abundance of at least one target polynucleotide has been reduced relative to a non-target polynucleotide, and where at least one target polynucleotide is any one of the 16 genes listed in the specification; (2) a kit for the enrichment of at least one low abundance polynucleotide in a sample of polynucleotides, where the sample comprises at least one high abundance polynucleotide and at least one low abundance polynucleotide, and where the kit comprises at least one enzymatically non-extendable nucleobase oligomer having a nucleobase sequence complementary to the high abundance target polynucleotide; (3) analyzing gene expression in a sample having at least one high abundance polynucleotide; (4) synthesizing a cDNA library enriched for at least one low abundance polynucleotide; and (5) enriching a sample for one or more low abundance polynucleotides.

BIOTECHNOLOGY - Preferred Method: In enriching low abundance polynucleotide relative to a high abundance polynucleotide in a sample, the ratio of the high abundance polynucleotide to the low abundance polynucleotide is at least 100:1. The sample comprises a first and a second high abundance polynucleotide and is exposed to at least two enzymatically non-extendable nucleobase oligomers, where one nucleobase oligomer comprises a nucleobase sequence that is complementary to a sequence within the first high abundance polynucleotide and the second nucleobase oligomer comprises a nucleobase sequence that is complementary to a sequence within the second high abundance polynucleotide. The low and high abundance polynucleotides are cDNA molecules or RNA molecules consisting of mRNA, rRNA, cRNA or tRNA molecules. The enzymatically non-extendable nucleobase oligomer does not have a ribose-containing oligomeric structure. The enzymatically non-extendable nucleobase oligomer is a peptide nucleic acid (PNA) oligomer, a modified nucleotide oligomer or internucleotide analog oligomer. The modified nucleotide oligomer is 2'-modified or 3'-modified nucleotide oligomer. The 2'-modified and 3'-modified nucleotide oligomers consist of 2'-O-alkyl modified nucleotide oligomers and 3'-alkyl modified nucleotide oligomers. Preferably, the 2'-O-alkyl modified nucleotide oligomers are 2'-O-methyl nucleotide oligomers. The modified nucleotide oligomer or internucleotide analog oligomer is selected from locked nucleic acids (LNA), N3'-P5' phosphoramidate (NP) oligomers, minor groove binder-linked-oligonucleotides (MGB-linked oligonucleotides), phosphorothioate (PS) oligomers, C1-C4 alkylphosphonate oligomers, phosphoramidates, beta-phosphodiester oligonucleotides, and alpha-phosphodiester oligonucleotides. The C1-C4 alkylphosphonate oligomers are methyl phosphonate (MP) oligomers. The enzymatically non-extendable first nucleobase oligomer is chimeric. The sample comprises more than one high abundance polynucleotide. The sample of polynucleotides comprises RNA or DNA. The RNA is mRNA, cRNA or total cellular RNA. The sample of polynucleotides comprises RNA, and polymerase **extension** is by reverse transcription to yield a first strand cDNA. The method further comprises second strand cDNA synthesis. The sample is exposed to at least one enzymatically non-extendable nucleobase oligomer during first and/or second strand cDNA synthesis. The method further comprises an amplification step by polymerase chain reaction or in vitro transcription. The sample of polynucleotides comprises DNA, and polymerase **extension** is by DNA-dependent DNA-polymerase in a polymerase chain reaction. The method further comprises labeling the amplified polynucleotides, which is concomitant with or subsequent to amplification. Enriching a sample for one or more low abundance

polynucleotides comprises amplifying the low abundance polynucleotides using polymerase **extension** while blocking amplification of at least one high abundance polynucleotide, where blocking amplification of the high abundance polynucleotide comprises contacting the high abundance polynucleotide prior to **amplification** with an **enzymatically** non-extendable oligomer comprising a sequence that is complementary to a sequence within the high abundance polynucleotide under conditions such that base pairing occurs, and where the ratio of the high abundance polynucleotide to each low abundance polynucleotide is at least 10:1. The sample is enriched for at least 10-100 low abundance polynucleotides. Amplification of at least 2-50 high abundance polypeptides is blocked. Preferably, the sample is enriched for at least 10 low abundance polynucleotides and the amplification of at least 2 high abundance polypeptides is blocked. Analyzing gene expression in a sample having at least one high abundance polynucleotide comprises exposing the sample to at least one enzymatically non-extendable nucleobase oligomer having a nucleobase sequence complementary to a sequence within the high abundance polynucleotide under conditions such that base pairing occurs, subjecting the sample to conditions for polymerase **extension** to produce an enriched polynucleotide sample, labeling the polynucleotides in the enriched polynucleotide sample, contacting the labeled polynucleotide sample with a **probe** using a **hybridization** means to form a **hybridization** complex, and detecting the **hybridization** complex, where the detection of a **hybridization** complex is indicative of gene expression. Synthesizing a cDNA library enriched for at least one low abundance polynucleotide comprises providing a sample of mRNA having at least one high abundance transcript and at least one low abundance transcript, exposing the sample to at least one enzymatically non-extendable nucleobase oligomer having a nucleobase sequence complementary to a sequence within the high abundance mRNA under conditions such that base pairing occurs, subjecting the sample to conditions for reverse transcription and first strand cDNA synthesis, subjecting the sample to conditions for second strand cDNA synthesis to form double stranded cDNA molecules, and cloning the double stranded cDNA molecules into a vector to yield an enriched cDNA library. Preferred Polynucleotide: The relative abundance of at least one non-target polynucleotide has been increased relative to a target polynucleotide. The polynucleotides are DNA molecules, preferably cDNA molecules, or RNA molecules, preferably cRNA molecules. The polynucleotides are labeled. The cDNA molecules are cloned into a vector. Preferred Kit: The sample comprises at least five high abundance polynucleotides, and the kit comprises at least five non-enzymatically non-extendable nucleobase oligomers each having a nucleobase sequence complementary to one of the five high abundance target polynucleotides. The kit additionally comprises a primer for amplifying the low abundance polynucleotide. The primer is a random primer. The high abundance target polynucleotide is any one of the 16 genes listed in the specification. The kit further comprises one or more components consisting of an RNA-dependent DNA polymerase (reverse transcriptase), a DNA-dependent RNA polymerase, a DNA-dependent DNA polymerase, an oligo-dT polymerase primer, an oligo-dT polymerase primer further comprising nucleotide sequence for RNA polymerase initiation, deoxyribonucleotide triphosphates, ribonucleotide triphosphates, a DNA polymerase primer for cDNA second strand synthesis, or a means for polynucleotide labeling.

USE - The methods are useful for the selective enrichment of low abundance polynucleotides in a sample. The enriched low abundance polynucleotides can be used in analyzing gene expression in a sample and creating cDNA libraries.

EXAMPLE - A total of 1 microg polyA mRNA isolated from human liver tissue was used in a 20 microl reverse transcription (RT) reaction containing at least one 2'-O-methyl ribonucleotide blocking oligomer that is capable of **hybridizing** to the beta-actin mRNA transcript. The RT reaction mixture was denatured at 70 degreesC for 5 minutes. First

strand cDNA synthesis was performed by the addition of 100-200 U reverse transcriptase, 1 mM dNTPs and 30 U RNase inhibitor, and incubated at 42 degreesC for 2 hours. The RT reaction was terminated by heating at 65 degreesC for 15 minutes. Second strand cDNA was synthesized using a DNA-dependent polymerase and random DNA primers. The resulting low abundance double-stranded cDNA was made blunt-ended by treatment with 10-20 U of T4 DNA polymerase for 15 minutes and 37 degreesC. Blunt-end, double-stranded cDNA was purified by filtration column or affinity capture column.(62 pages)

L6 ANSWER 2 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-14598 BIOTECHDS
TITLE: Method for detecting nucleic acid by **hybridization**, useful for detecting single-nucleotide polymorphisms, where sample and **probe** are reacted before immobilization; DNA sample and DNA **probe** immobilization for SNP detection
AUTHOR: FISCHER D; GEISTLINGER J
PATENT ASSIGNEE: IPK INST PFLANZENGENETIK and KULTURPFLANZE
PATENT INFO: DE 10245145 8 Apr 2004
APPLICATION INFO: DE 2002-1045145 27 Sep 2002
PRIORITY INFO: DE 2002-1045145 27 Sep 2002; DE 2002-1045145 27 Sep 2002
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 2004-331660 [31]
AN 2004-14598 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Method for detecting nucleic acid by: (a) mixing and **hybridizing** single-stranded (ss) sample molecules (NA1) with ss **probe** molecules (NA2), having a sequence at least partly complementary to NA1; (b) arranging and immobilizing the hybrids formed on a carrier; and (c) detecting **hybridization** by a standard method.

BIOTECHNOLOGY - Preferred Process: NA2, each of which represents a different locus, and NA1 from different individuals are analyzed in parallel. Up to 20000, preferably 96-384 NA1 and/or NA2 are analyzed. Single-nucleotide polymorphisms (SNP) are detected by enzymatic, template-dependent **extension** of NA2, which functions as a primer within the hybrid, with incorporation of a labeled nucleotide or analog (A). Preferably a chain-terminating (A) is introduced, specifically a dideoxynucleotide triphosphate. Preferred Materials; NA2 contains 10-100, best 20-30, nucleotides (nt) and NA1 contains 30-250, best 80-140, nt. NA1 are produced by PCR **amplification** and selective **enzymatic** degradation of a strand; by asymmetric PCR and/or affinity purification of the two amplified strands. NA2 are prepared by chemical synthesis.

USE - The method is specifically used to detect single-nucleotide polymorphisms, in pharmacogenomics or pharmacogenetics applications, including diagnosis, also generally for genotyping of humans, animals, microbes, fungi or plants, most particularly barley, Arabidopsis, rape and maize. Other applications are detecting microorganisms, analysis of transcriptional activity; and mutational, recombination, parentage and segregation analyses.

ADVANTAGE - The method allows detection of many SNPs, in many individuals, in parallel. It minimizes sources of error and does not require: (a) precise control over analytical parameters, e.g. sample concentration and equal **hybridization** temperatures; or (b) complex temperature cycling systems.

EXAMPLE - Analysis of the published data on the barley genome was used to identify 48 single-nucleotide polymorphisms (SNP) then primers designed that amplified genomic fragments containing these SNPs. One primer (5'-PTO; phosphorothioate) was complementary to the **extension** primer used later. Then 5'-amino-modified **probe** molecules were designed; they ended exactly 3' of the polymorphic site.

The amplified genomic fragments were incubated with the T7 5'-exonuclease to degrade the strand not protected by 5'-PTO; the **probe** molecules (i.e. the **extension** primers) were added; the mixture incubated; non-**hybridized** strands and nucleotides were removed and the double-stranded hybrids (in solution) used to produce a microarray on a commercial epoxy-coated microscope slide. An **extension** reaction was performed (50 degreesC for 1 hour) in presence of Cy5-labeled dideoxyadenosine triphosphate and R110-labeled dideoxyguanosine triphosphate; the slides washed and analyzed using a laser scanner to record only fluorescently labeled **extension** primers i.e. those that had been extended. The specification includes a photograph showing results at the 48 loci (all C/T polymorphisms) for the barley cultivars Morex and Barke.(21 pages)

L6 ANSWER 3 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-14786 BIOTECHDS
TITLE: Detecting nucleic acid, useful for diagnosis and prognosis of disease, comprises reacting an immobilized sequence with a cleavable, labeled **probe** then mass spectrometric detection;
DNA **probe** and DNA primer for SNP and multidrug-resistance gene mutation detection
AUTHOR: SCHATZ P; SCHUSTER M; BERLIN K
PATENT ASSIGNEE: EPIGENOMICS AG
PATENT INFO: DE 10240746 18 Mar 2004
APPLICATION INFO: DE 2002-1040746 1 Sep 2002
PRIORITY INFO: DE 2002-1040746 1 Sep 2002; DE 2002-1040746 1 Sep 2002
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 2004-331422 [31]
AN 2004-14786 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting nucleic acid (NA) by immobilization, reaction with a cleavable **probe** containing a mass marker, cleaving and mass spectrometric detection of the cleaved marker.

DETAILED DESCRIPTION - Method for detecting nucleic acid (NA) comprises: (1) binding at least one NA to a solid phase; (2) **hybridizing** NA sequence specifically to **probes** (P) that contain a cleavable bond and a (P)-specific mass marker (MM); (3) removing unhybridized (P); (4) contacting **hybridized** (P) with a matrix that both cleaves the bond and serves as matrix for MALDI MS (matrix-assisted laser desorption-ionization mass spectrometry) and (5) detecting MM at those positions where NA have bound. An INDEPENDENT CLAIM is also included for a kit comprising solid phase for immobilization, (P), components for performing MS measurements and instructions for performing the new method.

BIOTECHNOLOGY - Preferred Materials: NA are DNA, especially variant sequences that contain single nucleotide polymorphisms, point mutations, deletions, inversions or insertions, optionally pretreated, especially with bisulfite for detection of DNA methylation status at selected CpG positions. NA may be **amplified**, e.g. by **enzymatic** primer **extension**, PCR, rolling circle amplification or ligase chain reaction before being immobilized, especially with many different sequences being amplified in the same vessel. The carrier is a sample carrier for a mass spectrometer, especially for MALDI MS, and NA are deposited in a rectangular or hexagonal pattern. (P) are optionally modified DNA, locked NA, peptide NA or their hybrids, and after **hybridization** they may be modified by enzymatic primer **extension** or ligation, and may include at least one CG, TG or CA dinucleotide. Preferably MM is introduced during such modification and carries a single positive or negative charge. The MM used differ from each other by at least 1 D. Preferred Process: Amplified DNA is tested for **hybridization** with two types of (P): one binds preferentially to a sequence, derived from genomic DNA by chemical

treatment, where a C residue being tested is methylated, while the other binds to the corresponding sequence containing non-methylated C. Alternatively, one (P) recognizes either methylated or non-methylated C and the other binds independently of the methylation status. The test NA is derived from e.g. cell lines, blood, urine, other body fluids or paraffin-embedded tissue samples. Cleavage of the oligonucleotide is by treatment with an endo- or exo-nuclease.

USE - The method is used for diagnosis and/or prognosis of a wide range of conditions, e.g. side effects of pharmaceuticals; dysfunction, injury or diseases of the central nervous, cardiovascular, gastrointestinal, respiratory systems or skin; endocrine/metabolic or behavioral disorders; psychosis; dementia; inflammation; infections; or sexual dysfunction; also for investigating cell type, tissues and cell differentiation.

EXAMPLE - A genomic sequence was treated with bisulfite, desulfonated, then the promoter region of the MDR1 gene amplified (primer sequences given) to generate a 242 bp amplicon, 5'-modified by thiol. This was attached (using bromoacetic acid) to a polylysine-treated solid phase, and treated with two acid-labile oligonucleotides (sequences reproduced) designed to detect methylated or unmethylated C at position 198 of the amplicon. These oligonucleotides contained 5'-aminoadenosine, easily hydrolyzed with acid, and were linked, at 3'-amino, to 6-triethylammoniumhexyl N-hydroxysuccinimide ester or to its trimethyl analog, differing in mass by about 70 D. After **hybridization** with these oligonucleotides, the carrier was treated with 350 mM 3-hydroxypicolinic acid in acetonitrile containing 1.5% trifluoroacetic acid and the cleavage products were detected by matrix-assisted laser desorption-ionization mass spectrometry, to indicate methylation status at the specified C. (10 pages)

L6 ANSWER 4 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:121423 SCISEARCH
THE GENUINE ARTICLE: 767JY
TITLE: Single nucleotide polymorphism genotyping using locked nucleic acid (LNA((TM)))
AUTHOR: Mouritzen P (Reprint); Nielsen A T; Pfundheller H M; Choleva Y; Kongsbak L; Moller S
CORPORATE SOURCE: Exigon AS, New Technol Dev, Bygstubben 9, DK-2950 Vedbaek, Denmark (Reprint); Exigon AS, New Technol Dev, DK-2950 Vedbaek, Denmark
COUNTRY OF AUTHOR: Denmark
SOURCE: EXPERT REVIEW OF MOLECULAR DIAGNOSTICS, (JAN 2003) Vol. 3, No. 1, pp. 27-38.
Publisher: FUTURE DRUGS LTD, UNITEC HOUSE, 3RD FL, 2 ALBERT PLACE, FINCHLEYY CENTRAL, LONDON N3 1QB, ENGLAND.
ISSN: 1473-7159.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Locked nucleic acid (LNA(TM)) is a new class of bicyclic high affinity DNA analogs. LNA-containing oligonucleotides confer significantly increased affinity against their complementary DNA targets, increased mismatch discrimination (DeltaT(m)) and allow full control of the melting point of the **hybridization** reaction. LNA chemistry is completely compatible with the traditional DNA phosphoramidite chemistry and therefore LNA-DNA mixmer oligonucleotides can be designed with complete freedom for optimal performance. These properties render LNA oligonucleotides very well suited for SNP genotyping and have enabled several approaches for enzyme-independent SNP genotyping based on allele-specific **hybridization**. In addition, allele-specific PCR assays relying on enzymatically-enhanced discrimination can be improved using LNA-modified oligonucleotides. The use of LNA transforms

enzyme-independent genotyping approaches into experimentally simple, robust and cost-effective assays, which are highly suited for genotyping in clinical and industrial settings.

L6 ANSWER 5 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-08757 BIOTECHDS
TITLE: Detection device useful for detecting binding between members of specific binding pair, and for multiparallel thermal analysis of samples, has an array of addressable thermistors; the use of detection device for use in DNA, RNA and enzyme detection
AUTHOR: ROACH J S; WOLTER A
PATENT ASSIGNEE: PROLIGO LLC
PATENT INFO: WO 2002099386 12 Dec 2002
APPLICATION INFO: WO 2002-US18200 7 Jun 2002
PRIORITY INFO: US 2001-296685 7 Jun 2001; US 2001-296685 7 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-148685 [14]
AN 2003-08757 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A detection device (I) comprising an array of addressable thermistors (101), each closely associated to either a first member of a specific binding pair or to a binding or reaction partner to an analyte, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) preparing a detection device, by providing a solid support, and associating at least two thermistors with the solid support in an addressable array, connecting the thermistors and further associating at least one spot of a first member of a specific binding pair or reaction partner of known composition surrounding and in close proximity to the thermistors, wherein each thermistor is connected to a signal processor; and (2) an instrument for detecting a thermal event comprising (I), where each of the thermistors is interfaced to a processing unit, and a delivery device comprised of an injector, for introducing a substance to the detection device.

USE - (I) is useful for detecting binding between a first member of a specific binding pair and a second member of a specific binding pair, by providing (I), providing the first member of the specific binding pair, contacting (I) with a sample containing one or more of the second member of the specific binding pair, and detecting the binding between the binding pair members by thermal analysis. The binding pair is complementary nucleic acids, antibody/antigen, ligand/receptor, enzyme/substrate or aptamer/target. The specific binding pair comprises chimeric molecules, and the complementary nucleic acids are chosen from DNA/DNA, DNA/RNA, DNA/linear nucleic acid (LNA), DNA/short interfering RNA (siRNA) and DNA/peptide nucleic acid (PNA). The binding is the **hybridization** between the complementary nucleic acids. The first member of the specific binding pair is attached to the detection device by a covalent or non-covalent bond. The first member of the specific binding pair is localized over the detection device in the form of a solution that is in close proximity to the thermistor of the detection device. Each member of the binding pair contains a reactive moiety such as groups selected to maximize the heat of reaction. The binding between the binding pair members is a covalent reaction between the reactive moieties, or a non covalent interactions followed by covalent reaction between the reactive moieties on each member of the binding pair. The detection device provides a real time, digital profile of the binding between the binding pair members as is occurs in the detection device. (I) is also useful for detecting an analyte in a solution, by providing (I), providing a binding or reaction partner to the analyte, where the partner is closely associated with a spatially addressable thermistor in the detection device, contacting (I) with a sample containing one or more analytes, and detecting the binding or reaction between the analyte and

its binding or reaction partner by thermal analysis. The two molecules and the analyte each contain a reactive moieties. The reaction is a chemical ligation, and the binding between the analyte and its binding partner is used to distinguish between perfectly complementary and non-complementary sequences in which the non-complementary elements may comprise one or more elements of the mismatched sequence. The binding between the analyte and its binding partner comprises part of an **enzymatic amplification** reaction, especially polymerase chain reaction (PCR) or primer **extension** reaction. The detection device provides a real time, digital profile of the binding or reaction between the analyte and its binding or reaction partner. (All claimed.) (I) is useful for multiparallel thermal analysis of samples, particularly in the analysis of nucleic acids.

ADVANTAGE - The detection device monitors the binding events and reactions occurring in real time, the analysis time in detecting analytes is greatly reduced, and it is cost-effective. The post-incubation step is avoided, and it eliminates the need for labeling the **probe** molecules. (60 pages)

L6 ANSWER 6 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-02845 BIOTECHDS
TITLE: Analysis of methylation of cytosine bases in genomic DNA samples isolated from human sources, by utilizing bisulfite treatment and fluorescence polarization assay techniques; DNA primer, DNA **probe**, DNA chip and bioinformatics for high throughput screening and disease diagnosis
AUTHOR: BERLIN K; DISTLER J
PATENT ASSIGNEE: EPIGENOMICS AG
PATENT INFO: WO 2002061124 8 Aug 2002
APPLICATION INFO: WO 2002-EP923 29 Jan 2002
PRIORITY INFO: DE 2001-1004938 29 Jan 2001; DE 2001-1004938 29 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-674825 [72]
AN 2003-02845 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Analyzing methylation of cytosine bases in genomic DNA samples, comprising chemically treating nucleic acid sample to convert unmethylated cytosine to uracil, amplifying using primers specific for converted sequence, **hybridizing** amplificate with primers, extending primers by fluorophore labeled **probes** and polymerase, digesting the reaction solution and detecting fluorescence polarization of labeled nucleotides, is new.

DETAILED DESCRIPTION - Analyzing methylation of cytosine bases in genomic DNA samples, comprising: (a) the genomic DNA is chemically treated in such a manner that cytosine is converted into uracil or a similar base regarding the base pairing behavior in the DNA duplex, 5-methylcytosine however remains unchanged; (b) the chemically treated DNA is amplified using of at least one species of oligonucleotide (type A) as a primer in a polymerase reaction; (c) the amplificate is left in solution with one or more species of fluorophore labeled nucleotides and one or more species of oligonucleotide (type B), where the type B oligonucleotide **hybridizes** under appropriate conditions with its 3' end directly on or up to 10 bases from the position to be examined, and type B oligonucleotide is at least partly nuclease resistant; (d) the **hybridized** oligonucleotide (type B) is extended by a polymerase by at least one nucleotide, where the **extension** is dependent upon the methylation status of the respective cytosine position in the genomic DNA sample; (e) the solution is incubated with a phosphodiesterase, which is capable of digesting nucleic acids, however incompletely digests the type B oligonucleotides and its **extension** products; and (f) the fluorescence polarization of the solution is measured for each fluorescent label. An

INDEPENDENT CLAIM is also included for a diagnostic kit comprising one or more oligonucleotide primers designed to **hybridize** to bisulfite treated DNA sequence within 1-10 bases 3' of the target site, at least one species of nucleotides, where each species of nucleotide is covalently linked to a unique fluorophore, and a DNA polymerase that reacts with the oligonucleotide primer and nucleotides to produce a 3' **extension** of the primer.

BIOTECHNOLOGY - Preferred Method: All or a variable proportion of the fluorophore labeled nucleotides are dideoxynucleotides. The DNA sample is cleaved prior to bisulfite treatment with restriction endonucleases. After the polymerase amplification of the bisulfite DNA, the nucleotide of the polymerase reaction are diminished by phosphatase and the phosphatase is subsequently thermally denatured. The primer **extension** is detected by an increase in fluorescence polarization. The fluorophore is 5'carboxyfluorescein, 6-carboxy-X-rhodamine, BODIPY, Texas Red, DAPI, HEX, TET and N, N, N', N',-tetramethyl-6-carboxy- X-rhodamine, Cy3, Cy5, and fluorescein-isothiocyanate (FITC). The fluorescence polarization of the fluorophore labeled nucleotides and/or dideoxynucleotides is measured prior to incorporation into the DNA duplex and again after incorporation into the DNA duplex. The fluorescence polarization of the **enzymatically amplified** DNA is measured directly from the container in which the polymerase reaction was carried out. The type B primers are immobilized on a surface prior to **hybridization** with the amplificate. The bisulfite treated DNA is immobilized on a surface prior to **hybridization** with the fluorophore labeled nucleotides. The surface comprises silicon, glass, polystyrene, aluminum, steel, iron, copper, nickel, silver or gold. The information generated about the methylation status at the target site is provided to a computing device comprising one or more databases, or learning algorithms.

USE - The method is useful for analyzing the methylation status of cytosine bases in DNA samples isolated from human sources e.g. cell lines, blood, sputum, feces, urine, brain, cerebrospinal fluid, tissue embedded in paraffin, for e.g. tissue of eyes, intestine, kidney, brain, heart, prostate, lung, chest, liver and/or histological slides (claimed), for use in high throughput analysis, research or clinical settings. The method can be adapted to other diagnostic formats, for e.g. high density DNA chip analysis.

ADVANTAGE - The method allows analysis of the sequence in a closed tube, i.e. concurrent with or upon completion of the **enzymatic amplification** without need for further purification. The method is cost-effective and results are obtained minutes after carrying out the methylation specific reaction.

EXAMPLE - The methylation status of the genes ER1 and tumor necrosis factor (TNF) were analyzed using fluorescence polarization analysis of flourescently labeled nucleotides incorporated during a primer **extension** reaction. Double stranded DNA to be analyzed was bisulphite treated in order to convert unmethylated cytosine within the sample into thymidine, unmethylated cytosine remaining unaffected by the treatment. The bisulphite treated DNA was subsequently polymerase chain reaction (PCR) amplified and the purified PCR product was reamplified using asymmetric primer concentrations in order to amplify the G-rich (forward) strand. The single stranded template was analyzed using a primer **extension** reaction, where fluorescent labeled dATPs were incorporated at cytosine positions which had been unmethylated in the original DNA sample. Incorporation of dATP resulted in an increase in fluorescence polarization, therefore the degree of methylation within the DNA sample was inversely proportional to the degree of fluorescence polarization. Asymmetric PCR used primers: AGGAGGGGGAATTAAATAGA, and ACAATAAAACCATCCCAAATAC. The single stranded product was then analyzed in a primer **extension** reaction. Primer **extension** reaction were carried out within a BMG microplate. Fluorescein labeled dATPs were used in the primer **extension** reaction, incorporation

of the dATPs thus indicating the degree of methylation within the original DNA fragment. A mastermix containing water, dNTPs, buffer and dATP-fluorescein was prepared, the mix was distributed between the wells of the plate and primer and template DNA added. After this gain adjustment of the fluorescein polarization instrument was made and then the Klenow fragment was added. The reaction solution contained dNTP, dATP-fluorescein, primer (CAGGAAACAGCTATGACACAATAAAACCATCCCAAATAC), PCR product and Klenow. 4 different reaction solutions were compared. 1 was control and the other three (2, 3, 4) contained R74 ER1-B-L-M13b primer: CAGGAAACAGCTATGACACAATAAAACCATCCCAAATAC, R78 ER1-B-L-M13b-A TNF-beta-L: CAGGAAACAGCTATGACACAATAAAACCATCCCAAATAC, and R92 M13b-A: CAGGAAACAGCTATGACAAAAACCCCAAAATAAACAA, respectively. An increase in fluorescence polarization was observed in reaction mixtures 2, 3 and 4.(40 pages)

L6 ANSWER 7 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-01925 BIOTECHDS
TITLE: Novel polynucleotide library useful in molecular characterization of a carcinoma, comprising a pool of polynucleotide sequences or its subsequences which are either underexpressed or overexpressed in tumor cells; vector-mediated recombinant protein gene transfer and expression in host cell for use in mamma cancer prevention, diagnosis and therapy
AUTHOR: BERTUCCI F; HOULGATTE R; BIRNBAUM D; NGUYEN C; VIENS P; FERT V
PATENT ASSIGNEE: IPSOGEN
PATENT INFO: WO 2002046467 13 Jun 2002
APPLICATION INFO: WO 2001-IB2811 7 Dec 2001
PRIORITY INFO: US 2001-7926 7 Dec 2001; US 2000-254090 8 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-619023 [66]
AN 2003-01925 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A polynucleotide library (I) useful in molecular characterization of a carcinoma, comprising a pool of polynucleotides or its subsequences which are either underexpressed or overexpressed in tumor cells, and correspond to any of the polynucleotide sequences chosen from 468 sequences such as 317, 2584, 417, 1498, 423, 3327, 255 and 1325 bp fully defined in specification or its complement, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a polynucleotide array (II) useful for prognosis or diagnostic of tumor, comprising (I); and (2) detecting (M1) differentially expressed polynucleotide sequences which are correlated with a cancer, involves obtaining a polynucleotide sample from a patient, and reacting the polynucleotide sample obtained with a **probe** immobilized on a solid support, where the **probe** comprises any combination of the polynucleotide sequences of (I) or its expression products encoded by polynucleotide sequences of (I), and detecting the reaction product.

BIOTECHNOLOGY - Preferred Polynucleotide: In (I), the polynucleotide sequences or subsequences of the pool correspond to any combination of at least one polynucleotide selected from 50%, 75% or 100% of the predefined sets (212 sets fully defined in the specification). The sequences useful for differentiating a normal cell from a cancer cell is chosen from sets such as sets comprising (317 and 2584 bp), (300 and 369 bp). The sequences useful in detecting hormone sensitive cell is chosen from sets such as a set comprising (294, 362 and 2797 bp), or (359, 410 and 1356 bp). The sequences useful in differentiating a tumor with lymph nodes from a tumor without lymph nodes is chosen from sets such as set comprising (483 and 449 bp), (294, 362 and 2797 bp). The sequences useful in differentiating antracycline-sensitive tumors from antracycline-insensitive tumors is chosen from sets such as a set

comprising (294, 362 and 2797 bp), (438, 390 and 2475 bp). The sequences useful in classifying good and poor prognosis primary breast tumors is chosen from sets such as set comprising (439 and 1996 bp) and (397, 468 and 3064 bp) fully defined in the specification. The polynucleotides are immobilized on a solid support such as nylon membrane, nitrocellulose membrane, glass slide, glass beads, membranes on glass support or a silicon chip, to form a polynucleotide array.Preferred Method: In (M1), the polynucleotide sample is labeled before its reaction step, and the label is radioactive, colorimetric, **enzymatic**, molecular **amplification**, bioluminescent or fluorescent labels. The method further comprises obtaining a control polynucleotide sample, reacting the control sample with the **probe**, detecting a control sample reaction product and comparing the amount of the polynucleotide sample reaction product to the amount of the control sample reaction product. The polynucleotide sample is cDNA, RNA or mRNA, where mRNA is isolated from the polynucleotide sample and cDNA is obtained by reverse transcription of the mRNA. The reaction step is performed by **hybridizing** the polynucleotide sample with the **probe**.
The product encoded by any of the polynucleotide sequences or polynucleotide sequences sets is involved in a receptor-ligand reaction on which detection is based. The polynucleotide sample is obtained from a patient treated with the anti-tumor agent to be screened.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Anti-tumor agent. No biological data given.

USE - (I) is useful in molecular characterization of a carcinoma. (I) and (II) are useful for prognosis or diagnostic of tumor, in differentiating a normal cell from a cancer cell, detecting hormone sensitive tumor cell, differentiating a tumor with lymph nodes from a tumor without lymph nodes, differentiating antracycline-sensitive tumors from antracycline-insensitive tumors, and classifying good and poor prognosis primary breast tumors. (M) is useful for detecting, diagnosing, staging, monitoring, or predicting conditions associated with cancer, especially breast cancer. (M) is also useful for detecting differentially expressed polynucleotide sequences during the prevention or treatment of conditions associated with cancer, especially breast cancer. (M) is also useful for screening an anti-tumor agent, where the polynucleotide sample is obtained from a patient treated with the anti-tumor agent (claimed). (I) is useful for large-scale molecular characterization of breast cancer that help in prediction, prognosis and cancer treatment, and for detecting differentially expressed genes that correlated with a cancer.

EXAMPLE - Molecular characterization of a carcinoma was performed as follows. Total RNA from normal breast tissue was obtained and RNA was isolated. cDNA arrays were prepared that contained polymerase chain reaction (PCR) products of 5 control clones, and 180 IMAGE human cDNA clones selected with practical criteria. The control clones were Arabidopsis thaliana cytochrome c554 gene, 3 poly(A) sequences of different sizes and the vector pT7T3D (negative controls). Five ng of total RNA from tissue samples were used for each labeling. **Probes** were prepared from total RNA with an excess of oligo (dT25) to saturate the poly(A) tails of the messengers, and to insure that the reverse transcribed product did not contain long poly(T) sequences. A precise amount of c554 mRNA was added to the total RNA before labeling to allow normalization of the data. **Hybridization** was done in excess of target (15 ng of DNA in each spot) and binding of cDNAs to the targets was linear and proportional to the quantity of cDNA in the **probe** . Quantitative data were obtained using an imaging plate device. **Hybridization** signal detection with a FUJI BAS 1500 machine and quantification with the HDG analyzer software were done. Quantification was done by integrating all spot pixel intensities and subtracting a spot background value determined in the neighboring area. Spots were located with a LaPlacian transformation. Quantified data were normalized and expressed as absolute gene expression levels. The data represented that the values in each sample displayed a wide range of intensities corresponding to expression levels ranging from 0.002%-5% of mRNA

abundance. Absolute values were log-transformed omitting 18 clones whose median intensity was equal to zero across all tissues. Data for each of the 162 remaining clones were then median-centered, as well as data for each sample, so that the relative variation was known. A hierarchical clustering program was then applied to group the 35 samples according to their overall gene expression profiles, and to group the 162 clones on the basis of similarity of their expression levels in all tissues. The clustering algorithm identified two groups of samples, designated A (n = 15, including normal breast, NB) and B (n = 20). These groups were similar with respect to patient age, menopausal status at diagnosis, SBR grading and tumor pathological size. 72% of tumors in group A were node-positive and 75% in group B were node-negative. 80% of the tumors in group B were estrogen receptor (ER) positive and 50% in group A were ER-negative. In the group A of 15 samples, three samples were different from each other and from the other 12 samples. The latter constituted two subgroups of tumors, A1 and A2. 32 genes out of 188 were identified by comparing their median expression level in A1 vs A2. Then, the 12 tumors were reclustered using the expression profiles of these genes. High expression of v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2), v-myc avian myelocytomatosis viral oncogene homolog (MYC) and epidermal growth factor receptor (EGFR) was associated with bad prognosis subgroup A1 (6-8), and that of E-cadherin and the proto-oncogene v-myb avian myeloblastosis viral oncogene homolog (MYB) with good prognosis subgroup A2. To identify genes differentially expressed between breast tumors (T) and normal breast (NB), the NB value for each gene was compared to its expression level in each tumor. When the expression level of a gene in NB was undetectable, only qualitative information was deduced and the mRNA was considered as differentially expressed if the signal intensity in the tumor was superior to the reproducibility threshold (0.002% of mRNA abundance). Also, the number of tumors where it was over- or underexpressed was measured. The T/NB ratio which represented median expression level in 34 breast tumors/expression level in normal breast, ranged from 2.7 ATP-binding cassette (ABCC5) to 17.76 gata-binding protein 3 (GATA3) for the overexpressed genes, and from 0.00 (desmin) and 0.29 adenomatosis polyposis coli (APC) for the underexpressed genes. MYB-like2 transcript displayed a median expression level of 0.025% in breast tumors and was undetectable in NB. High expression of mucin 1, ERBB2, fibroblast growth factor receptor1 (FGFR1) and FGFR2, v-myc avian myelocytomatosis viral oncogene homolog, stromelysin 3, cathepsin D and downregulation of v-fos fbj murine osteosarcoma viral oncogene homolog, APC, b-cell c11/lymphoma 2 (BCL2) were found. To search for genes whose expression profile was correlated with axillary lymph node status, a strong prognostic factor in breast cancer, the group of node-negative tumors was compared with the group of tumors with massive axillary **extension**. The gene encoding the tyrosine kinase receptor ERBB2 was the most significantly overexpressed gene in node-positive tumors and displayed the highest correlation coefficient. Gene clustering showed groups of genes with correlated expression across samples. Correlation coefficients between gene pairs in the 34 tumors were often high. Highly correlated gene expression was that of BCL2 and RBL2. Furthermore, these genes also exhibited significant correlated expression with other genes such as PPP2CA, AKT2, PRKCSH or TNFRSF6/FAS. In particular, a striking correlated expression between BCL2 and FAS was observed. These results highlighted the great potential of cDNA array in cancer research. The gene expression profiles conformed the heterogeneity of breast cancer.(401 pages)

L6 ANSWER 8 OF 16 MEDLINE on STN
ACCESSION NUMBER: 96406412 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8810545
TITLE: A simplified method of HLA-DR typing with the PCR method using the genomic DNA extracted from blood-absorbed filter.
AUTHOR: Kabayama H
CORPORATE SOURCE: 2nd Department of Pediatrics, Ohashi Hospital, Toho

University School of Medicine.
SOURCE: Nihon Rinsho Men'eki Gakkai kaishi = Japanese journal of clinical immunology, (1996 Jun) 19 (3) 201-9.
Journal code: 9505992. ISSN: 0911-4300.
PUB. COUNTRY: Japan
DOCUMENT TYPE: (CLINICAL TRIAL)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961205

AB The **enzymatic amplification** of specific DNA sequences by the polymerase chain reaction (PCR) has provided a new approach to genetic typing of HLA-class II region specificities. However, we found the existing PCR protocols to be adequate and/or time consuming, especially when large numbers of samples need to be processed. We have devised a simplified method which alleviates these **problems**. Genomic DNA prepared from peripheral blood nucleated cells was facilitated using the punched-out filter paper absorbed with the peripheral blood. Second exon HLA-DRB segments of the genomic DNA were amplified by the polymerase chain reaction (PCR) (30 cycles: denaturing at 94 C for 1 min; annealing at 55 for 1 min, **extension** at 72 C for 2 min) using HLA-DRB primer DRB 5'-1 (ACCGGTCGTTCITGTCCCCICAGCA) and DRB 3'-1 (CTCGCCICTGCACIGTIAAGC) designed in our laboratory. The presence of specific alleles in a PCR-amplified sample was determined by dot-blot **hybridization** with 32 P-labeled sequence-specific-oligonucleotides (SSOs). Quantitation of the producer is being evaluated using an automated scanner. These newly designed primers allowed amplification of all the known expressed alleles of DRB 1, B 3, B 4 and B 5 loci. This type of reproducible and precise assay is very useful in the HLA-class II typing of large number of patients.

L6 ANSWER 9 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 93:321220 SCISEARCH
THE GENUINE ARTICLE: LC393
TITLE: PRIMER **EXTENSION** ANALYSIS PROVIDES A SENSITIVE TOOL FOR THE IDENTIFICATION OF PCR-AMPLIFIED DNA FROM HIV-1
AUTHOR: BONI J (Reprint); SCHUPBACH J
CORPORATE SOURCE: UNIV ZURICH, INST MED VIROL, NATL CTR RETROVIRUSES, GLORIASTR 30, CH-8028 ZURICH, SWITZERLAND (Reprint); SWISS FED OFF PUBL HLTH, SWISS NATL CTR RETROVIRUSES, ZURICH, SWITZERLAND
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: JOURNAL OF VIROLOGICAL METHODS, (MAY 1993) Vol. 42, No. 2-3, pp. 309-322.
ISSN: 0166-0934.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Primer **extension** analysis was evaluated as a means to identify PCR-amplified DNA from HIV-1. Solution **hybridization** with radioactive labeled oligonucleotides followed by an **extension** reaction with Klenow fragment of Escherichia coli DNA polymerase I and subsequent separation on denaturing polyacrylamide gels reveals single stranded DNA products of the predicted size. The specificity of the reaction is further demonstrated by specific endonuclease digestion. The analysis is more sensitive than Southern blotting and about equally sensitive as Slot blot analysis when double-stranded DNA **probes**

are used for **hybridization**. With end-labeled oligonucleotide **probes**, primer **extension** analysis proved an order of magnitude more sensitive than membrane **hybridization**. The analysis also allows quantitation of amplified DNA from 1 pg to about 1 ng of DNA product. Under the conditions described for amplification, primer **extension** analysis is capable of detecting a single HIV-1 plasmid DNA molecule in the presence of 1 mug of total DNA. 3'-end mismatching of the oligonucleotide **probe** does not result in a significantly altered detection limit. Primer **extension** analysis can also be carried out with at least three different PCR-amplified DNAs in the same reaction tube.

L6 ANSWER 10 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 93:5565 SCISEARCH
THE GENUINE ARTICLE: KE060
TITLE: DETECTION OF SPECIFIC ALLELES BY USING ALLELE-SPECIFIC PRIMER **EXTENSION** FOLLOWED BY CAPTURE ON SOLID SUPPORT
AUTHOR: UGOZZOLI L; WAHLQVIST J M; EHSANI A; KAPLAN B E; WALLACE R B (Reprint)
CORPORATE SOURCE: CITY HOPE NATL MED CTR, BECKMAN RES INT, DEPT MOLEC BIOCHEM, DUARTE, CA, 91010; CITY HOPE NATL MED CTR, BECKMAN RES INT, DEPT MOLEC GENET, DUARTE, CA, 91010
COUNTRY OF AUTHOR: USA
SOURCE: GENETIC ANALYSIS-TECHNIQUES AND APPLICATIONS, (AUG 1992) Vol. 9, No. 4, pp. 107-112.
ISSN: 1050-3862.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This article describes a method for determining whether a particular nucleic acid sequence is present in a sample and for discriminating between any two nucleic acid sequences if such sequences differ only by a single nucleotide. The method entails **extension** of a novel two-component primer on templates that may or may not include a target nucleic acid sequence. The 3' portion of the primer is complementary to a portion of the template adjacent to the target sequence (for example, the polymorphic nucleotide). The 5' portion of the primer is complementary to a different preselected nucleic acid sequence. **Extension** of the 3' portion of the primer with a labeled deoxynucleoside triphosphate yields a labeled **extension** product, but only if the template includes the target sequence. The presence of such a labeled primer-**extension** product is detected by **hybridization** of the 5' portion to the preselected sequence. The preselected sequence is immobilized on a solid support. The method has been applied to genotyping individuals for the two-allele polymorphism of the human tyrosinase gene.

L6 ANSWER 11 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 91:214109 SCISEARCH
THE GENUINE ARTICLE: FF039
TITLE: USE OF INOSINE-CONTAINING OLIGONUCLEOTIDE PRIMERS FOR **ENZYMATIC AMPLIFICATION** OF DIFFERENT ALLELES OF THE GENE CODING FOR HEAT-STABLE TOXIN TYPE-I OF ENTEROTOXIGENIC ESCHERICHIA-COLI
AUTHOR: CANDRIAN U (Reprint); FURRER B; HOFELEIN C; LUTHY J
CORPORATE SOURCE: UNIV BERN, INST BIOCHEM, FOOD CHEM LAB, FREIESTR 3, CH-3012 BERN, SWITZERLAND (Reprint)
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1991) Vol. 57, No. 4, pp. 955-961.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A method which employs the polymerase chain reaction (PCR) to identify Escherichia coli strains containing the estA gene was developed. This gene codes for heat-stable enterotoxin type I. The use of an inosine-containing pair of amplification primers allowed the amplification of a specific 175-bp DNA fragment from several different estA alleles. The amplified fragments were identified and distinguished by allele-specific oligonucleotide **hybridization** and characterized by restriction endonuclease analysis. An **extension** of the classical two-primer PCR proved to be a very simple and rapid method to identify and characterize the estA alleles. Besides the inosine-containing pair of primers, which recognized all described alleles, additional oligonucleotides were used as primers. The sequence of each of these primers was allele specific, and each was amplification compatible with one of the inosine-containing primers. Thus, in one PCR the 175-bp fragment typical for all estA alleles and an allele-specific fragment of different size were produced. These fragments could be separated by agarose gel electrophoresis and were recognized by ethidium bromide staining. Twenty-seven E. coli strains were tested with this amplification system. The presence or lack of the genetic information for production of heat-stable enterotoxin type I was perfectly consistent with the ability of these strains to produce this enterotoxin, as determined by enzyme-linked immunosorbent assay.

L6 ANSWER 12 OF 16 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 91:52208 SCISEARCH
THE GENUINE ARTICLE: ET526
TITLE: THE OLIGOMER **EXTENSION** HOT BLOT - A RAPID ALTERNATIVE TO SOUTHERN BLOTS FOR ANALYZING POLYMERASE CHAIN-REACTION PRODUCTS
AUTHOR: PARKER J D; BURMER G C (Reprint)
CORPORATE SOURCE: UNIV WASHINGTON, SCH MED, DEPT PATHOL, SM-30, SEATTLE, WA, 98195
COUNTRY OF AUTHOR: USA
SOURCE: BIOTECHNIQUES, (1991) Vol. 10, No. 1, pp. 94.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 19

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report a modification of a liquid **hybridization** method that serves as a rapid, sensitive alternative to Southern **hybridization** for the analysis of polymerase chain reaction (PCR) products. An aliquot of the completed PCR is mixed with an internally nested, end-labeled oligonucleotide **probe**, and one cycle of PCR is performed. The products are electrophoresed, dried and analyzed by autoradiography. The method is significantly more sensitive than standard Southern **hybridization** methods, is equally specific, requires as little as 1-10 picograms of **probe** and results can be obtained in less than an hour. The procedure is equally useful for identifying products in multiplex PCRs or single-stranded PCRs.

L6 ANSWER 13 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-03708 BIOTECHDS
TITLE: **Enzymatic amplification** and detection of nucleic acid;
DNA amplification, RNA amplification with high sensitivity by **hybridization**
PATENT ASSIGNEE: Du-Pont

PATENT INFO: WO 8910979 16 Nov 1989
APPLICATION INFO: WO 1989-US1996 10 May 1989
PRIORITY INFO: US 1988-192090 10 May 1988
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1989-356504 [48]
AN 1990-03708 BIOTECHDS
AB A nucleic acid **probe** assay for detection of a preselected nucleic acid is new. The assay comprises making the target DNA single-stranded and amplifying at least 1 nucleic acid sequence by treatment with 2 different primers under conditions suitable for **extension**. The primers are sufficiently complementary to the different strands to be amplified so that they **hybridize** with them. When the **extension** product is separated from its complement, it can serve as an additional template for synthesis of the **extension** product or the other primer. Primer **extension** products are separated from their templates to produce single-stranded molecules. Single-stranded molecules are treated with primers for further rounds of amplification. The products are contacted with capture and reporter **probes** in solution. The sandwich product produced is contacted with a solid support having a surface group capable of reacting with the anchor group of the capture **probe**. Unhybridized reporter **probe** is removed and the immobilized reporter group is detected and/or measured. The method allows **enzymatic amplification** of a nucleic acid sequence and high detection sensitivity. (30pp)

L6 ANSWER 14 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1989-13582 BIOTECHDS
TITLE: Direct detection of herpes virus DNA sequences in clinical samples by in vitro **enzymatic amplification**
;
DNA amplification using the polymerase chain reaction (conference abstract)
AUTHOR: Rowley A H; Wolinsky S M
LOCATION: Department of Pediatrics and Medicine, Division of Infectious Diseases, Northwestern University Medical School, Children's Memorial Hospital and Northwestern Memorial Hospital, Chicago, IL, USA.
SOURCE: J.Cell.Biochem.; (1989) Suppl.13E, 302
CODEN: JCEBD5
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1989-13582 BIOTECHDS
AB Oligonucleotide primers and **probes** from a region of the DNA-polymerase (EC-2.7.7.7) gene conserved among herpes viruses were designed for use in **enzymatic amplification** using the polymerase chain reaction. Clinical samples were lysed and treated with nonionic detergents and protease-K, and amplified for 30 cycles of thermal denaturation (94 deg), primer annealing (55 deg) and Taq DNA-polymerase-directed primer **extension** (72 deg). An aliquot of amplified product was **hybridized** in solution to a 32P-labeled oligonucleotide **probe**; the target DNA:**probe** heteroduplex was resolved on a 6% polyacrylamide gel followed by autoradiography. Generic primers were used to amplify herpes simplex virus types 1 and 2, Epstein-Barr virus and cytomegalo virus; a type-specific **probe** uniquely identified each virus. A different primer pair and **probe** specifically identified varicella-zoster virus DNA. Multiple strains of virus were detected from infected cells, cell-free supernatants and blood using this technique. This method will be useful in the rapid diagnosis of herpes virus infections and in the detection of new herpes viruses. (0 ref)

L6 ANSWER 15 OF 16 MEDLINE on STN

ACCESSION NUMBER: 88246483 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3288864
TITLE: Detection of toxigenic Escherichia coli using biotin-labelled DNA **probes** following **enzymatic amplification** of the heat labile toxin gene.
AUTHOR: Olive D M; Atta A I; Setti S K
CORPORATE SOURCE: Department of Microbiology, Faculty of Medicine, Kuwait University.
SOURCE: Molecular and cellular probes, (1988 Mar) 2 (1) 47-57. Journal code: 8709751. ISSN: 0890-8508.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198807
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880718

AB Several types of DNA **probes** labelled with biotin were compared for their sensitivity to detect the heat labile toxin (LT) gene in toxigenic Escherichia coli. In addition, a procedure was developed for enzymatically amplifying LT gene sequences in toxigenic E. coli. **Probes** were labelled with biotinylated nucleotides by either nick translation; 3' tailing; primer **extension** of **probe** DNA cloned into bacteriophage M13; sandwich **hybridization**; or oligolabelling of isolated DNA fragments. A single stranded **probe** consisting of a DNA fragment from the LT gene cloned into the bacteriophage M13mp18 and detected by **hybridization** to oligolabelled biotinylated M13mp18 RF DNA in a sandwich **hybridization** was able to detect as little as 10 pg of toxin gene DNA. Cloned LT gene DNA was serially diluted and **amplified enzymatically** using synthetic oligonucleotide primers. Amplified DNA was detected using biotin-labelled M13-based **probes**. As little as 1 fg of LT DNA could be amplified to detectable levels by this method. Experiments with LT+ bacteria resulted in the detection of as few as 1000 bacteria. The combination of **enzymatic amplification** coupled with M13-based DNA **probes** provides a highly sensitive tool for detecting pathogenic microorganisms.

L6 ANSWER 16 OF 16 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1987-00252 BIOTECHDS
TITLE: Analysis of **enzymatically amplified** beta-globin and HLA-DQ-alpha DNA with allele-specific oligonucleotide **probes**;
DNA amplification using the polymerase chain reaction method
AUTHOR: Saiki R K; Bugawan T L; Horn G T; Mullis K B; Erlich H A
CORPORATE SOURCE: Cetus
LOCATION: Department of Human Genetics, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, California 94608, USA.
SOURCE: Nature; (1986) 324, 6093, 163-66
CODEN: NATUAS
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1987-00252 BIOTECHDS
AB Allele-specific oligonucleotide (ASO) **probes** have been used in the analysis of genetic diseases caused by single base mutations. To improve the sensitivity, specificity and simplicity of this method, the polymerase chain reaction (PCR) procedure was used to amplify a specific segment of the beta-globin or HLA-DQ-alpha gene in human genomic DNA before **hybridization** with ASOs. The PCR primers, 20 bases in length, anneal to opposite strands of the genomic DNA flanking the target sequence. The primers are positioned so that the polymerase-catalyzed,

template-directed **extension** of 1 primer can serve as a template strand for the other. Repeated cycles of denaturation, primer annealing and primer **extension** result in the exponential accumulation of the DNA fragment. The amplification method gives an over 100,000-fold increase in target sequence, permitting the analysis of allelic variation in 1 ng of genomic DNA using a simple blot for **probe hybridization**. PCR amplification was also used directly on crude cell lyzates, eliminating the need for DNA purification. (17 ref)